INTERNATIONAL
AYURVEDIC
MEDICAL JOURNAL

IAMJ

Research Article ISSN: 2320 5091 Impact Factor: 5.344

EVALUATION OF ANTIBACTERIAL ACTIVITY OF ANISOMELES INDICA (LINN.) KUNTZE LEAF

Priyanka Singh[1*], Lalchand Sahu[1], Rajesh Kumar Singh[2]

[1]M.D. Scholar, [2]Lecturer,
Post Graduate Department of Dravyaguna, Govt. Ayurvedic College Raipur, Chhattisgarh, India

Email: drlal4ayurved@gmail.com

ABSTRACT

The past decade has witnessed a significant increase in the prevalence of resistance to antimicrobial agents. Resistance to antimicrobial agents has important implications for morbidity, mortality and health care costs in hospital, as well as in the community. Hence, substantial attention has been focused on to minimize usage of antimicrobial drugs and develop plant origin antimicrobial drugs for the treatment of microbial infections. *Anisomeles indica* (Linn.) Kuntze belongs to Lamiaceae family commonly known as 'Indian Catmint' and its vernacular names *Kala bhangra, Gobara, Chodharo, Gopali* etc. The antimicrobial screening of the hydroalcoholic extract of *A.indica* was investigated by the Cup plate (cylinder plate) diffusion method and Turbidimetric test (MIC and MBC/MFC) method. Results of this study revealed a remarkable ZOI. The extract was shown the maximum ZI of 32 mm for *E.Coli* & *St. pyogens*, 31 mm for *S.aureus* and 28 mm for *P.aeruginosa* at a concentration of 100 µl. The obtained MIC values for hydroalcoholic extract of *A.indica* 200 to 25 µl against *E.coli,* 200 to 50 µl against *P.aeruginosa* & *St. pyogens* and 200 to 75 µl extract against *S. aureus.* The obtained MBC value of hydoalcoholic extract of *A.indica* 200 to 75 µl against *E.coli and St. pyogens*. 200 to 100 µl against *P.aeruginosa* & *S. aureus.* The MBC values obtained for the extract against the pathogens are higher than MIC, indicating that the extract is bacteriostatic at lower concentrations and bactericidal at higher concentrations. When the activity of the extract was compared with control antibiotic Ceftriaxone, it was found to be significant and this confirms that the selected plant has good antibacterial potential. The results indicate the leaves of *A.indica* have a potential broad spectrum antibacterial activity and this extract can be used as formulation to treat the infectious diseases caused by the test organisms.

Keywords: *Anisomeles indica*, Indian Catmint, Antibacterial.

INTRODUCTION

Ancient Indian, Chinese, Unani, Egyptian manuscripts described several plant species of therapeutics importance. The Ayurveda medicine system have become significantly more popular all over the globe, because of the curative property, minimal side effects, availability in the vicinity and having many more

preparation of a drug according to disease & patients. According to the World Health Organization (W.H.O) report, more than 80% of world population is still dependent on herbal remedies to treat illness[1]. Since ancient times, man attributed efforts for the search of drug from natural compounds especially plants. Currently approximately 25% of drug derived from plants and many other are synthetic analogues built on prototype compounds isolated from plant species in modern pharmacopoeia[2]. The people of modern generation are picking up the traditional knowledge from their ancestors on the basis of observation only. Many of these prescriptions are very effective because they are based on years of experience. From the literature it reveals that State with rich flora has remained botanically almost unexplored 68. Pathogenic bacteria cause bacterial infection, whereas the other does not. Nonpathogenic are commonly called normal flora. Some species of bacteria are opportunistic pathogens and causes disease mainly in people suffering from immune suppression[3]. Newer antibacterial have increased therapeutic options and hence leading to demand for in vitro determination of antibacterial susceptibility of some drugs of plants origin. Therefore, there is need to develop more effective and safe plant origin antibacterial agents[4].

The purpose of this research was to develop the safe and more active plant origin antibacterial drugs for the treatment of infectious diseases. In past few decades some modern antimicrobial drugs developed resistance, some of them having less effect on microbes and causing adverse effects on human being. Ultimately, our primary concern regarding resistance is that resistant bacteria are more difficult to get rid of and that complications and deaths resulting from infections caused by them will increase with time. Very few really new antibiotics have been developed recent years term, and tradition natural sources of antibiotics[5]. Plants represent an inexhaustible source of novel chemical compounds, which are of potential use in medicine and other applications. Plants consist of many active compounds such as alkaloids, steroids, tannins, glycosides, volatile oils, fixed oils, resins, phenols and flavonoids, which found in their specific parts such as leaves, flowers, bark, seeds, fruits, root, etc. The beneficial medicinal effects of plant materials typically result from the combination of these secondary products. These compounds selectively inhibit the function of biological targets[6].

Anisomeles indica (Linn.) Kuntze belongs to Lamiaceae family commonly known as 'Indian Catmint' and its vernacular names *Kala bhangra, Gobara, Chodharo, Gopali* etc. It is used in folk medicine in the treatment of diverse condition such as inflammatory skin diseases, liver diseases, intestinal infection, abdominal pain and immune system deficiencies[7]. The present study deals with its In vitro Antibacterial activity against four bacterial strains.

Materials & Methods

In vitro antibacterial and antifungal activities were examined for hydroalcoholic extract of *Anisomeles indica*. Antibacterial and antifungal activities of plant extract against four pathogenic bacteria (*Escherichia coli, Pseudomonas aeruginos, Staphylococcus aureus, Streptococcus pyogens*) strains were investigated by the Cup plate (Cylinder plate) diffusion method and turbidimetric test (MIC and MBC/MFC) method[8].

Preparation of the plant extract: The powdered drug (100 gm) in thimble was placed in Soxhlet extractor and extracted with ethanol and distilled water (70:30) for 72 hours at the temperature of 40°C. The solutions were filtered using Whatman filter paper. The filtered hydro-alcoholic extract of *A. indica* leaf was used for antimicrobial screening.

Preparation of Microbial Culture medium: Culture medium or growth medium is a solid, liquid or semi-solid. Culture medium contains nutrients and physical growth parameters necessary for microbial growth. All microorganisms cannot grow in single culture medium. The Nutrient broth for bacteria, Nutrient Agar (NA) medium for bacteria, Sabouraud's dextrose agar medium for fungi (SDA), Muller hinton agar medium (MHA), Brain heart infusion broth (BHI) media were used to culture the microorganisms.

All the media were sterilized in Autoclave at 15 lb/120° C for 15 minutes. The glassware was sterilized in Hot air oven at 180° C for two hours. Approximately 20 ml of the media were added to each 180 mm x

20 mm sterile Petri dishes. Slants were used to maintain the pure cultures to enable the required number of microorganisms for assay. The master cultures were maintained carefully in sterile condition during subculture.

Microbial strains for the antimicrobial study: The standard microorganisms were obtained from the Himedia Company Maharashtra, India. The bacterial strains were grown in the nutrient broth and maintained on nutrient agar slants at 4° C. Following four bacterial strains has been procured from Himedia Company for the present study.

Cup Plate diffusion method: In cup plate method, the antimicrobial substance diffuse from the cup through a solidified agar layer in a plate to an extent so that the growth of added microorganisms is inhibited entirely in a circular area or zone around the cavity containing the solution of a known quantity of antimicrobial substance. The antimicrobial activity is expressed as the zone of inhibition in millimeters, which is measured with a zone reader.

The Prepared Muller hinton agar medium plates were inoculated with the test organism and then with the help of a sterile borer cavities were made which later were filled with the described concentrations (5, 10, 25, 50, 75, and 100 µl) of the extracts and standard antibacterial dispensed with the help of sterile micropipette. Then plates were incubated at 37 °C for 24 hours for bacterial culture and for 48 hours at 27 °C for fungal culture before taking the reading. After incubation the zone of inhibition were measured. A sterile borer was used to prepare dig cups of 6 mm diameter in the agar medium spread with the microorganisms and 0.1 ml of inoculum was spread on the agar plate by spread plate technique. Accurately measured solution of each concentration and reference standards were added to the cups with micropipette. The hydroalcoholic extract of *A. indica* was screened for antibacterial activity against a wide spectrum of bacteria and the activity was compared with appropriate reference standards (Ceftriaxone for Gram positive, Gram negative bacterial strains and Fluconazole for fungal strains).

Turbidimetric or tube assay method

The turbidimetric assay to detect the drug potency is based on inhibition of microbial growth as indicated by measurement of the turbidity (transmittance) of suitable micro-organisms in a fluid medium to which have been added, graded amounts of the test compounds and known concentration of reference material. This includes Determination of Minimal inhibitory concentration (MIC) & Minimal bactericidal / fungicidal concentration (MBC/MFC).

Determination of Minimal inhibitory concentration (MIC)

Requirements: Brain heart infusion (BHI) broth, Assay tubes, Test organisms, Micropipettes, Vortex mixer, Test drug and standard drugs.

Method: The MIC of the plant extracts is determined according to the micro broth dilution method (Murray et al.,1996). It is performed in test tubes (ependroff tubes) for determining the minimum inhibitory concentration (MIC). Standardized suspensions of the test organisms are inoculated into a series of tubes, including one growth (Positive control) and one sterility control (Negative control). For dilution of the extract, different concentration of the plant extract is added to the mixture of BHI (brain heart infusion) broth and the test organism. The concentrations of the plant extract used in the study included 200, 150, 125, 100, 75, 50, 25, 12, 10 and 5 µl. Then test tubes were incubated at 37 °C for 24 hours for bacterial culture and for 48 hours at 27 °C for fungal culture before taking the reading. After incubation these test tube will be observed for turbidity. The test tube showing the minimum turbidity will be noted for MIC.

Minimal bactericidal / fungicidal concentration (MBC/MFC)

The MBC and MFC are the lowest concentration of the antimicrobial agent required to kill microorganism. The MBC and MFC are determined using a series of steps, undertaken after a MIC test has been completed. The MBC and MFC are determined by first selecting tubes that showed no growth during MIC determination; incubated for further 24 hours at 37 °C for bacterial culture and for 48 hours at 27 °C for fungal culture. The least concentration, at which no

growth is observed, is noted and considered as the MBC and MFC.

Procedure

1. Dispense 800 µl BHI broth to all the test tubes.
2. Dispense the 100 µl fresh culture to all the test tubes.
3. Dispense different concentrations (200, 150, 125, 100, 75, 50, 25, 12, 10 and 5 µl) of the drug extract to all the tubes except PC and NC.
4. Read the results after 24 hours and 48 hours for MIC: check for presence or absence of turbidity and not down the readings.
5. Proceed to MBC by plating a loopful of sample from all the test tubes on Nutrient agar plates and incubate for 24 hours for bacterial culture and 48 hours fungal culture.
6. After incubation read the reading.

RESULTS & DISCUSSION

A. Zone of inhibition (ZOI) by Cup Plate Method

The hydroalcoholic extracts of leaf with different volumes were transferred to the cylindrical plates and Zone of inhibition was observed & measured in mm.

Table 1: ZOI of hydroalcoholic extract of *A. indica* leaf against bacterial strains

S. No. →	**1**	**2**	**3**	**4**	**5**	**6**	**7**
Organism / Concentration	100	75	50	25	10	5	ND
Escherichia coli	32mm	29mm	27mm	22mm	18mm	14mm	NZ
Pseudomonas aeruginosa	28mm	27mm	24mm	19mm	14mm	8mm	NZ
Staphylococcus aureus	31mm	29mm	27mm	24mm	14mm	7mm	NZ
Streptococcus pyogens	32mm	30mm	28mm	24mm	16mm	8mm	NZ

(Volume – µl**,** ND – No drug, NZ – No Zone of Inhibition around the extract)

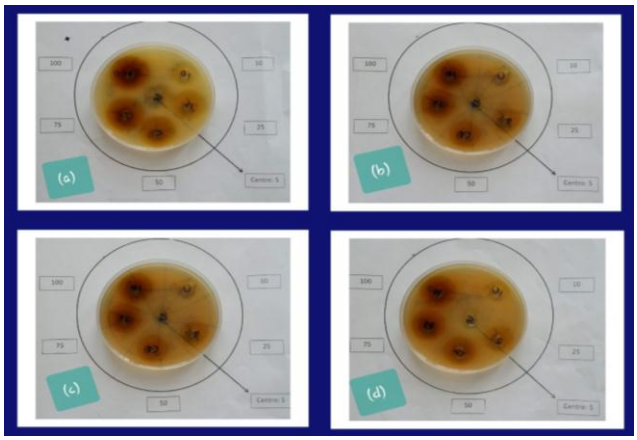

Fig.1: ZOI (Cup plate method) formed by hydroalcoholic extract of *A.indica* leaf at dff. Conc. Against (a) E.coli (b) P. aeruginosa (c) S. (d) St. pyogens

Table 2: ZOI of Standard drug Ceftriaxone against bacterial strains

S. No. →	**1**	**2**	**3**	**4**	**5**	**6**	**7**
Organism / Concentration	100	75	50	25	10	5	ND
Escherichia coli	41mm	38mm	34mm	31mm	18mm	NZ	NZ
Pseudomonas aeruginosa	45mm	43mm	39mm	36mm	34mm	NZ	NZ
Staphylococcus aureus	36mm	33mm	29mm	24mm	22mm	14mm	NZ
Streptococcus pyogens	44mm	42mm	38mm	36mm	32mm	18mm	NZ

(Concentration – µl**,** ND – No drug, NZ – No Zone of Inhibition around the drug)

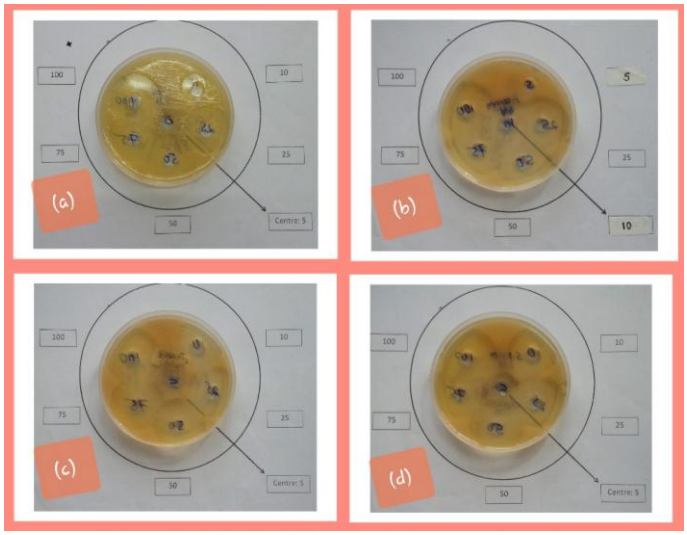

Fig.2: ZOI (Cup plate method) formed by Ceftriaxone at dff. Conc. Against (a) E.coli (b) P. aeruginosa (c) S. aureus (d) St. pyogens

The antibacterial potential of the extract was found to dose dependent. The *A.indica* hydroalcoholic extract was shown the minimum ZOI of 14 mm for *E.Coli*, 8 mm for *St. pyogens* & *P. aeruginosa*, 7 mm for *S. aureus* at a concentration of 5 µl and the maximum zone inhibition of 32 mm for *E.Coli* and *St. pyogens*, 31 mm for *S. aureus* and 28 mm for *P. aeruginosa* at a concentration of 100 µl.

When the activity of extract was compared with positive control (Ceftriaxone), it was found to be significant and this confirms that the selected plant has antibacterial potential. The antibacterial activity of the extract might be attributed to the presence of the secondary metabolites in the extract.

B. Minimum inhibitory concentration (MIC)

The effectiveness of the extract in tested bacterial strains was determined by measuring the MIC. MIC was performed for only those organisms which showed a ZOI and were sensitive to the plant extract in the previous antimicrobial assay by the Cup plate method.

Table 3: MIC of hydroalcoholic extract of *A.indica* leaf against bacterial strains

S. No. →	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**PC**	**NC**
Organism / Concentration	200	150	125	100	75	50	25	12	10	5	ND	No Organism
Escherichia coli	NT	NT	NT	NT	NT	NT	NT	T	T	T	T	NT
Pseudomonasaeruginosa	NT	NT	NT	NT	NT	NT	T	T	T	T	T	NT
Staphylococcus aureus	NT	NT	NT	NT	NT	T	T	T	T	T	T	NT
Streptococcus pyogens	NT	NT	NT	NT	NT	NT	T	T	T	T	T	NT

(Antibacterial activity – MIC, Concentration – **µl,** ND – No drug, PC – Positive Control, NC – Negative Control, NT – No turbidity, T – Turbidity)

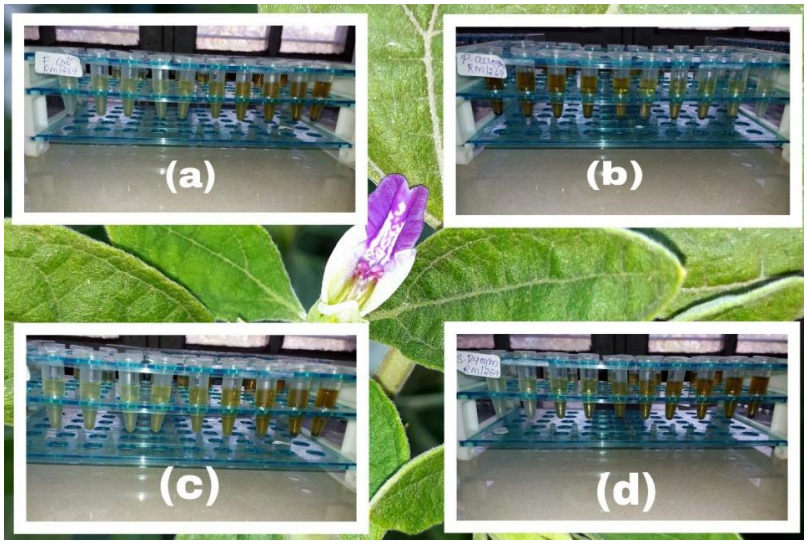

Fig.3: MIC of *A.indica* leaf at dff. conc. against (a) E.coli (b) P. aeruginosa (c) S. (d) St. pyogens

Table 4: MIC of Standard drug Ceftriaxone against bacterial strains

S. No. →	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**PC**	**NC**
Organism/Concentration	200	150	125	100	75	50	25	12	10	5	ND	No Organism
Escherichia coli	NT	NT	NT	NT	NT	NT	NT	NT	NT	T	T	NT
Pseudomonas aeruginosa	NT	NT	NT	NT	NT	NT	NT	NT	T	T	T	NT
Staphylococcus aureus	NT	NT	NT	NT	NT	NT	NT	NT	T	T	T	NT
Streptococcus pyogens	NT	NT	NT	NT	NT	NT	NT	NT	NT	T	T	NT

(Antibacterial activity – MIC, Concentration – **µl,** ND – No drug, PC – Positive Control, NC – Negative Control, NT – No turbidity, T – Turbidity)

The obtained MIC values of 200 µl, 150 µl, 125 µl, 100 µl, 75 µl, 50 µl and 25 µl for hydroalcoholic extract of A.indica leaves against E.coli. The obtained MIC values of 200 µl, 150 µl, 125 µl, 100 µl, 75 µl and 50 µl against for the extract P.aeruginosa & St. pyogens. The obtained MIC values of 200 µl, 150 µl, 125 µl, 100 µl and 75 µl for the extract against S. aureus.

The MIC was recorded if the solution did not appear turbid of bacterial accumulation when compared with the negative control and standard drug Ceftriaxone.

Minimum bactericidal concentration (MBC)

Table 5: MBC of hydroalcoholic extract of *A.indica* leaf against bacterial strains

S. No. →	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**PC**	**NC**
Organism/Concentration	200	150	125	100	75	50	25	12	10	5	ND	No Organism
Escherichia coli	NG	NG	NG	NG	NG	G	G	G	G	G	G	NG
Pseudomonasaeruginosa	NG	NG	NG	NG	G	G	G	G	G	G	G	NG
Staphylococcus aureus	NG	NG	NG	NG	G	G	G	G	G	G	G	NG
Streptococcus pyogens	NG	NG	NG	NG	NG	G	G	G	G	G	G	NG

(Antibacterial activity – MBC, Concentration – **µl,** ND – No drug, PC – Positive Control, NC – Negative Control, NG – No growth, G – Growth)

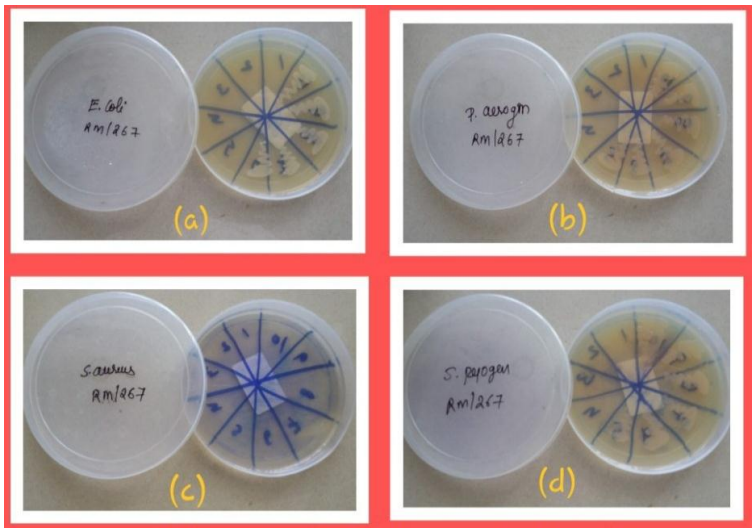

Fig.4: MBC of *A.indica* leaf at dff. conc. against (a) E.coli (b) P. aeruginosa (c) S. (d) St. pyogens

Table 6: MBC of Standard drug Ceftriaxone against bacterial strains

S. No. →	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**PC**	**NC**
Organism/Concentration	200	150	125	100	75	50	25	12	10	5	ND	No Organism
Escherichia coli	NG	NG	NG	NG	NG	NG	NG	G	G	G	G	NG
Pseudomonasaeruginosa	NG	NG	NG	NG	NG	NG	NG	NG	NG	G	G	NG
Staphylococcus aureus	NG	NG	NG	NG	NG	NG	NG	NG	NG	G	G	NG
Streptococcus pyogens	NG	NG	NG	NG	NG	NG	NG	NG	NG	G	G	NG

(Antibacterial activity – MBC, Concentration – **µl,** ND – No drug, PC – Positive Control, NC – Negative Control, NG – No growth, G – Growth)

The MBC was confirmed by absence of bacterial growth of the tested strains streaked form inhibition zone correspondence their lowest MIC's. *A.indica* extract showed potentially bactericidal activity against the tested pathogenic bacteria *E.coli, St. pyogens* with MBC values of 200, 150, 125, 100 & 75 µl, while both bacteria was less sensitive at 50, 25, 12, 10 and 5 µl doses. The extract showed potentially bactericidal activity against the tested pathogenic bacteria *P.aeruginosa, S. aureus* with MBC values of 200, 150, 125 & 100 µl, while both bacteria were less sensitive at 75, 50, 25, 12, 10 and 5 µl doses. In the present study the plant extract was bacteriostatic at lower concentration but bactericidal at higher concentration.

CONCLUSION

The result of this study concluded that all the tested bacteria were found to be highly susceptible to the hydro-alcoholic extract of *A.indica* leaf. The results of our study suggested that leaves of *A. indica* possess significant antibacterial activity. A further study of the extracts will be needed to isolate, characterize and elucidate the structure of the bioactive compounds present which were responsible for potent antimicrobial activity. However, it necessitates further extensive molecular and cellular level investigations to evaluate the therapeutic effect of phytochemicals present in *A. indica* and to identify its mechanism of action, following which it can serve as a valuable therapeutic option for bacterial infections.

REFERENCES

1. Kirtikar K.R, B.D. Basu, Indian Medicinal Plants,Vol. III, (International Book Distributors, Dehradun, India, 1999) 2212-2214.
2. Shaheen AF, Sheikh Ali Ahmad, Masood Rabbani, Asim Aslam, Tasra Bibi, Fakhra Liaqat, Javed Muhammad and Fatima Rehmani. Antibacterial activity of herbal extracts against MDR resistant *E.coli* recovered from retail chicken meat. Pak. J. Pharm. Sci. 2015; 28(4):pp. 1295-1300.
3. Entenza, J. M., Betrisey, B., Manuel, O., Giddey, M., Sakwinska, O., Laurent, F., & Bizzinib, A. Rapid detection of *staphylococcus aureus* strains with reduced susceptibility to vancomycin by isothermal micro calorimetry. Journal of Clinical Microbiology. 2014; 52(1): 180–186.
4. Pfaller Michael A. Antifungal drug resistance: Mechanisms, epidimeology and consequences for treatment. The American Journal of medicine. 2012; 125(1): S3-S13.
5. Carlos, F. (2010). Global perspectives of Antibiotic resistance. US Springer Science and Business Media. Pp.3-14.
6. Gupta Ankit, Narniwal Madhu and Kothari Vijay. Modern extraction methods for preparation of bioactive plant extracts. International Journal of Applied and Natural Sciences (IJANS) 1 Aug 2012; 1(1): 8-26.
7. Baranwal VK, Irchhaiya R, Singh S. *Anisomeles indica*-an overview. IRJP. 2012; 3:84-87.
8. Quality control methods for herbal drugs. World health organization. Updated edition of Quality control methods for medicinal plant materials, 1998.2011.

Source of Support: Nil
Conflict of Interest: None Declared

How to cite this URL: Priyanka Singh et al: Evaluation Of Antibacterial Activity Of Anisomeles Indica (Linn.) Kuntze Leaf. International Ayurvedic Medical Journal {online} 2020 {cited February, 2020} Available from: http://www.iamj.in/posts/images/upload/2746_2753.pdf